FOAMY VIRAL VECTOR COMPOSITIONS AND METHODS FOR THE MANUFACTURE OF SAME

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation of U.S. patent application Ser. No. 16/562,578 entitled "Foamy Viral Vector Compositions and Methods for the Manufacture of Same," filed Sep. 6, 2019, which is a continuation of U.S. patent application Ser. No. 15/041,087 entitled "Foamy Viral Vector Compositions and Methods for the Manufacture of Same," filed Feb. 11, 2016, now abandoned, which claims the benefit of and priority to U.S. Ser. No. 62/127,956, filed Mar. 4, 2015, of same title, in its entirety for all purposes.

GOVERNMENT RIGHTS

[0002] This invention was made with government support under HL070871, HL085107, and TR000077 awarded by NIH. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Foamy virus (FV) vectors are a promising alternative to gamma-retroviral and lentiviral vectors, demonstrating high transduction rates (1, 2) with less genotoxicity (3, 4). Additional advantages include the fact that the FV envelope has tropism for most cell types, the vector can carry larger expression cassettes as compared to gamma-retroviral and lentiviral vectors, the vector particles have increased stability due to a DNA genome formed in developing vector particles as compared to RNA, and FV is not associated with disease in humans (5). Combined, these properties make FV vector system the ideal candidate for gene therapy application. Proof of principle on the use of FV vectors for genetic correction was provided by Bauer et al. (6, 7) who demonstrated cure of dogs suffering from canine Leukocyte Adhesion Deficiency (LAD). The study used autologous CD34+ cells transduced with FV vector carrying the CD18 gene driven by the Murine Stem Cell Virus (MSCV) promoter. In 4-7 years of follow-up, there has been no emergence of clonal dominance or leukemia, supporting the claim that FV vectors are safe for clinical application.

[0004] To date, there has not been a concerted effort, published or unpublished, to generate, purify and highly concentrate FV vector particles for clinical application. Given the advantageous properties of FV, the ability to increase the titer of FV translates into practical benefits. Thus, there is a need in the art for improving titer of FV and methods for large-scale production of FV. The instant application addresses one or more such needs in the art.

BRIEF SUMMARY

[0005] Disclosed herein are methods of preparing FV vector particles, particularly to increase titer. The methods may comprise, in some aspects, the steps of transfecting a population of eukaryotic cells by contacting the population of eukaryotic cells with one or more transfection reagents to form a transfection mixture, and incubating the transfection mixture to form a transfected cell population; harvesting the FV vector particles from the transfected cell population, wherein the harvesting step may be carried out about 70 hours to about 100 hours, or about 70 hours to about 90 hours, or about 70 hours to about 80 hours, or about 72 hours to about 75 hours, post-transfection; purifying the FV vector particles; and concentrating the FV vector particles.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] FIG. **1** depicts a graph showing that optimal titer of FV vector requires the presence of 10% Fetal Bovine Serum (FBS). Transfection of 293T with FV-GFP using Calcium phosphate. Titer on HT1080 (Avg±SD, duplicate).

[0007] FIG. **2** depicts a graph showing that different lots of Fetal Bovine Serum (FBS) generate a different amount of FV vector transfection of 293T with FV-GFP using Calcium phosphate. Titer on HT1080 (Avg±SD, triplicate).

[0008] FIG. **3** depicts a graph showing the effect of a lipid supplement (Gibco Chemically-Defined Lipid Concentrate, Catalog #11905-031) in serum-free and low-serum media (D2, DMEM with 2% FBS; D10, DMEM with 10% FBS). Results show that DMEM with 10% FBS is superior (Avg±SD, duplicate).

[0009] FIG. **4** depicts a graph showing the effect of concentration of DMSO on the recovery of infectious FV vector after storage at −80° C. This data demonstrates that reduction in the amount of DMSO reduces the recovery of infectious virus.

[0010] FIG. **5** depicts a graph showing a comparison of the standard Calcium Phosphate (CaPhos) transfection method vs. PolyPlus "PEIPro" Transfection Reagent at various amounts (25 to 80 microLiter) per 10 cm dish, using a Foamy GFP vector. The data show increased titer with PEI as compared to Calcium Phosphate (Avg±SD, triplicate).

[0011] FIG. **6** depicts a graph showing a comparison of FV vector titer derived from transfection of 293T cells with calcium phosphate and PEI (PolyPlus PEIPro) at different amounts (volume PEI used per 10 cm tissue culture dish equivalent). Volume of 70 microLiter PEI per plate is optimal (Avg±SD, duplicate).

[0012] FIG. **7** depicts a graph showing a comparison of two methods of adding PEI compatible with large scale manufacturing: (1) mixing of cells with PEI and plasmid prior to plating; and (2) mixing of PEI and plasmid with media added to adherent cells. Controls are calcium phosphate and PEI using lab-scale methods. Results show that mixing PEI with media but not cells provides results similar to the Optimal PEI method (Avg±SD, triplicate).

[0013] FIG. **8** depicts a graph showing a comparison of complexion time of plasmid and PEI showed 10 minutes to be optimal as compared to 15 or 20 minutes. Data show titer of FV-GFP (Avg±SD, triplicate).

[0014] FIG. **9** depicts a graph showing a comparison of plates pre-seeded 3 days prior to transfection with PEI vs. the standard 1 day, using either existing media or fresh media for transfection, showed plating of 1 day prior to PEI transfection with fresh media to be optimal. Titers FV-GFP (Avg±SD, triplicate).

[0015] FIG. **10** depicts a graph showing the effect of different concentrations of optimized Gag plasmid (pCiGAGopt) on the titer of FV vector as compared to the standard amount (10.4 microgram) of non-optimized Gag (pCIGSAN). Data show a 5-fold improvement in titer using 16-fold diluted pCiGAGopt. Titers FV-CD18 (Avg±SD, duplicate).

[0016] FIG. **11** depicts a graph showing FV Titer generated by transfection with PEI without and with media change 19 hours post-transfection. The data show that more